

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Previously presented): A method for producing a recombinant glycoprotein in a unicellular or filamentous fungus host cell that lacks  $\alpha$ 1,6-mannosyltransferase activity, the method comprising the steps of diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure, and introducing into the host cell one or more nucleic acids molecules encoding an  $\alpha$ 1,2-mannosidase catalytic domain fused to a targeting peptide that targets the endoplasmic reticulum (ER) or Golgi apparatus in the host cell (ii) a GlcNAc transferase I (GnT I) catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell, and (iii) the recombinant glycoprotein,

wherein said method results in the production within the host cell of recombinant glycoproteins having *N*-glycans attached thereto comprising GlcNAcMan<sub>X</sub>GlcNAc<sub>2</sub> core structures, wherein X is 3 or 4.

Claim 2 (Previously presented): The method of claim 1, wherein the host cell further includes a nucleic acid molecule encoding a mannosidase II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and the method results in the production within the host cell of recombinant glycoproteins having *N*-glycans attached thereto comprising GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub> core structures.

Claim 3 (Previously presented): The method of claim 2, wherein the host cell further includes a nucleic acid molecule encoding a GnT II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and the method results in the production within the host cell of recombinant glycoproteins having *N*-glycans attached thereto comprising GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> core structures.

Claims 4-5 (Cancelled)

Claim 6 (Previously presented): The method of claim 1 wherein the host cell further includes a nucleic acid molecule encoding a GnT II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and the method results in the production within the host cell of recombinant glycoproteins having *N*-glycans attached thereto comprising GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> core structures.

Claim 7 (Previously presented): The method of claim 6, wherein the wherein the host cell further includes one or more nucleic acid molecules encoding one or more enzyme activities selected from galactosyltransferase, sialyltransferase, fucosyltransferase, and GlcNAc transferase III, IV, V, and VI.

Claim 8 (Previously presented): The method of claim 1, wherein at least one diminished or depleted enzyme is selected from the group consisting of an enzyme having dolichyl-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,3 mannosyltransferase activity; an enzyme having dolichyl-P-Man:Man<sub>6</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,2 mannosyltransferase activity and an enzyme having dolichyl-P-Man:Man<sub>7</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,6 mannosyltransferase activity.

Claim 9 (Previously presented): The method of claim 1, wherein the diminished or depleted enzyme has dolichyl-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,3 mannosyltransferase activity.

Claim 10 (Previously presented): The method of claim 1, wherein the enzyme is diminished or depleted by mutation of a host cell gene encoding the enzymatic activity.

Claim 11 (Previously presented): The method of claim 10, wherein the mutation is a partial or total deletion of a host cell gene encoding the enzymatic activity.

Claim 12 (Previously presented): The method of claim 1, wherein the attached *N* glycans have seven or fewer mannose residues.

Claim 13 (Canceled)

Claim 14 (Previously presented): The method of claim 1, wherein the glycoprotein comprises one or more sugars selected from the group consisting of galactose, GlcNAc, sialic acid, and fucose.

Claim 15 (Previously presented): The method of claim 1, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.

Claim 16 (Cancelled)

Claim 17 (Previously presented): The method of claim 1, wherein the unicellular or filamentous fungus host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum* and *Neurospora crassa*.

Claims 18-58 (Cancelled)

Claim 59 (Previously presented): A method for producing a human-like glycoprotein in a unicellular or filamentous fungus host cell comprising the steps of diminishing or depleting from the host cell an *alg* gene activity and introducing into the host cell at least one glycosidase activity.

Claims 60-65 (Cancelled)

Claim 66 (Previously presented): A method for producing a recombinant glycoprotein in a yeast host cell, the method comprising

(a) providing a yeast host cell in which the activity of an  $\alpha$ -1,6-mannosyltransferase and the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure have been diminished or depleted, and which comprises one or more nucleic acid molecules encoding

(i) an  $\alpha$ 1,2-mannosidase catalytic domain fused to a targeting peptide that targets the endoplasmic reticulum (ER) or Golgi apparatus in the host cell,

(ii) a GlcNAc transferase I (GnT I) catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell, and

(iii) a recombinant glycoprotein; and

(b) growing the host cell under conditions to produce the recombinant glycoprotein, wherein the recombinant glycoprotein has *N*-glycans attached thereto comprising GlcNAcMan<sub>X</sub>GlcNAc<sub>2</sub> core structures, wherein X is 3 or 4.

Claim 67 (Previously presented): The method of claim 66, wherein the host cell further includes a nucleic acid molecule encoding a mannosidase II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and wherein the recombinant glycoprotein that is produced has *N*-glycans attached thereto comprising GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub> core structures.

Claim 68 (Previously presented): The method of claim 67, wherein the host cell further includes a nucleic acid molecule encoding a GnT II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and wherein the recombinant glycoprotein that is produced has *N*-glycans attached thereto comprising a GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> structure.

Claim 69 (Previously presented): The method of claim 66, wherein the host cell further includes a nucleic acid molecule encoding a GnT II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and wherein the recombinant glycoprotein that is produced has *N*-glycans attached thereto comprising a GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> structure.

Claim 70 (Previously presented): The method of claim 66, wherein the host cell further includes one or more nucleic acid molecules encoding one or more sugar transporters selected from UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, and CMP-sialic acid transporter.

Claim 71 (Previously presented): The method of claim 66, wherein the host cell further includes one or more nucleic acid molecules encoding at least one enzyme activity selected from galactosyltransferase, sialyltransferase, fucosyltransferase, and GlcNAc transferase III, IV, V, and VI.

Claim 72 (Previously presented): The method of claim 66, wherein at least one diminished or depleted enzyme is selected from the group consisting of an enzyme having dolichyl-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,3 mannosyltransferase activity; an enzyme having dolichyl-P-Man:Man<sub>6</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,2 mannosyltransferase activity and an enzyme having dolichyl-P-Man:Man<sub>7</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,6 mannosyltransferase activity.

Claim 73 (Previously presented): The method of claim 66, wherein the diminished or depleted enzyme has dolichyl-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,3 mannosyltransferase activity.

Claim 74 (Previously presented): The method of claim 66, wherein the diminished or depleted enzyme activity is by mutation of a host cell gene encoding the enzymatic activity.

Claim 75 (Previously presented): The method of claim 74, wherein the mutation is a partial or total deletion of a host cell gene encoding the enzymatic activity.

Claim 76 (Previously presented): The method of claim 66, wherein the glycoprotein comprises one or more sugars selected from the group consisting of galactose, GlcNAc, sialic acid, and fucose.

Claim 77 (Previously presented): The method of claim 66, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.

Claim 78 (Previously presented): The method of claim 66, wherein the yeast host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, and *Candida albicans*.

Claim 79 (Previously presented): The method of claim 66, wherein the yeast host cell is *Pichia pastoris*.

Claim 80 (Previously presented): A method for producing a recombinant glycoprotein in a yeast host cell, the method comprising

(a) providing a yeast host cell in which the  $\alpha$ -1,6-mannosyltransferase activity and dolichyl-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-dolichyl alpha-1,3 mannosyltransferase activity of the host cell have been diminished or depleted, and which comprises one or more nucleic acid molecules encoding

- (i) an  $\alpha$ 1,2-mannosidase catalytic domain fused to a targeting peptide that targets the endoplasmic reticulum (ER) or Golgi apparatus in the host cell,
  - (ii) a GlcNAc transferase I (GnT I) catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell, and
  - (iii) a recombinant glycoprotein; and
- (b) growing the host cell under conditions to produce the recombinant glycoprotein, wherein the recombinant glycoprotein has *N*-glycans attached thereto comprising GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub> core structures.

Claim 81 (Previously presented): The method of claim 80, wherein the host cell further includes a nucleic acid molecule encoding a GnT II catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell and wherein the recombinant glycoprotein that is produced has *N*-glycans attached thereto comprising a GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> structure.

Claim 82 (Previously presented): The method of claim 80, wherein the host cell further includes one or more nucleic acid molecules encoding one or more sugar transporters selected from UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, and CMP-sialic acid transporter.

Claim 83 (Previously presented): The method of claim 80, wherein the host cell further includes one or more nucleic acid molecules encoding at least one enzyme activity selected from galactosyltransferase, sialyltransferase, fucosyltransferase, and GlcNAc transferase III, IV, V, and VI.

Claim 84 (Previously presented): The method of claim 80, wherein the diminished or depleted enzyme activity is by mutation of a host cell gene encoding the enzymatic activity.

Claim 85 (Previously presented): The method of claim 84, wherein the mutation is a partial or total deletion of a host cell gene encoding the enzymatic activity.

Claim 86 (Previously presented): The method of claim 80, wherein the glycoprotein comprises one or more sugars selected from the group consisting of galactose, GlcNAc, sialic acid, and fucose.

Application No.: 10/500,240  
Amendment Date: 22-Oct-10  
Reply to Office Action of: 14-Sep-10

Claim 87 (Previously presented): The method of claim 80, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.

Claim 88 (Previously presented): The method of claim 80, wherein the yeast host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, and *Candida albicans*.